Original Communications.

BACTERIOLOGICAL STUDY IN THE ÆTIOLOGY OF YELLOW FEVER.*

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ASSISTED BY
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THE recent visitation of yellow fever in New Orleans during the summer and fall of 1897 afforded us a splendid opportunity of studying from a bacteriological standpoint the causation of this disease. The published researches of Sternberg and Sanarelli were of great advantage to us. We followed as closely as we found practicable the technique of these experimenters, and we were not long in making discoveries and reaching a definite conclusion.

The news of the outbreak first reached us in mid-ocean, when casually glancing over a copy of the *New York Herald* of September 7th, belonging to a fellow-passenger. From that time on we had no other idea but the reaching as speedily as possible of our native city, to be on the ground and help the good cause—the study of this dreadful malady—and contribute our mite toward stamping it out forever. We reached New Orleans on September 28th, and forthwith began our labor in the pathological department of the Isolation Hospital and in the Bacteriological Laboratory which the Tulane Medical College so generously puts at the service of the Louisiana State Board of Health to carry on its work.

And here it might be best to begin by mentioning those who have been of assistance to us in our important and difficult task. First, we owe thanks to Dr. J. D. Bloom, house surgeon of the Charity Hospital, for his kind permission to have free access at all times to the Isolation Hospital and its post-mortem house; to Dr. J. H. Veazie, the visiting physician of the Yellow-Fever Hospital, for his kindness and, at times, valuable help; to Dr. Hamilton P. Jones, resident physician of the institution, for help in various ways, such as collecting blood slides for us, assisting us at autopsies, in taking notes and making our cultures from the blood and organs, and occasionally, when we were absent from the autopsies, in making cultures and forwarding same to us; to Dr. O. L. Pothier, pathologist of the Charity Hospital, for his uniform courtesy and kindness in the post-mortem house, making the autopsies at times to suit our convenience best, furnishing us with materials and specimens of organs, and allowing us to take cultures as we suggested and preferred; to Dr. R. S. Woodson, captain United States army, a daily attendant and worker in the laboratory, for his help in making plate cultures, performing comparative tests with various bacteria, under our guidance and at our suggestion, doing a great part of the work of the Widal's test, keeping our cultures alive, assisting us in the animal inoculations and at their autopsies, and in making cultures from them, and in the latter part of his stay with us, in making the animal autopsies for us; to Dr. Ott Lerch, for sundry services, too long to enumerate; to Dr. John Callan and other frequent visitors to the laboratory, for their kind encouragement; lastly, to Dr. John J. Archinard, a young brother and assistant, for the remarkable foresight with which he collected the blood of patients, made autopsies and cultures, and kept the organs of all his cases before our return, and after we had assumed charge, for his constant devotion and assistance, doing whatever we required of him always cheerfully and well, never caring how much humdrum or drudgery there might be in it all, provided it was our wish and for the benefit of science and mankind.

Our work may properly be divided into four parts, all of which are distinct, but which have enough relation together to be presented in the same treatise:

I. SEARCH FOR AND ISOLATION OF THE SPECIFIC GERM OF YELLOW FEVER IN THE CADAVERS OF YELLOW-FEVER PATIENTS, IN THE BLOOD OF THE SICK, AND IN THEIR EXCRETIONS.—For the purpose of avoiding repetition and to show clinically and pathologically the type of our most severe cases of yellow fever, we give below the history and post-mortem notes of one of these, closely observed from beginning to end:

N. B., taken sick September 30, 1897; slight chill; severe headache; anorexia and great pain in muscles of back and limbs; temperature, 103.6° F.; pulse, 140.

October 1st.—Temperature, 104.4° F.; pulse, 126; tongue very much coated; injected conjunctivæ; great nausea; urine highly colored; specific gravity, 1.020; scanty, thirteen ounces and a half in twenty-four hours; acid reaction; no albumin; diarrhœa, with dark-colored stools; blood examined for plasmodium; negative result.

2d.—Conjunctivæ more injected; peculiar facies particularly noticeable; muscular pain continuing; great nausea; severe headache; urine, twenty-two ounces in twenty-four hours; specific gravity, 1.020; reaction acid; albumin moist, five per cent.; no plasmodium; temperature, 104.4° F.; pulse, 98.

3d.—General symptoms remain same; albumin, ten per cent.; great gastric irritability; pain in the epigastrium; temperature, 104.4° F.; pulse, 84.

4th.—Great gastric irritability; pulse weak and slow, from 76 to 96; temperature, 105.2° F.; marked diminution of urine; albumin, thirty per cent.; slight black vomit.

5th.—Stage of calm; temperature, 102.2° F.; pulse,

* This article was intended for the meeting of the American Medical Association in Denver. Owing to the outbreak of the war the author foresaw that it would be impossible for him to attend said meeting, and he therefore read the article before the Louisiana State Medical Society, in New Orleans, on May 12, 1898.

88; almost complete suppression of urine; albumin, sixty per cent.; beginning jaundice; black vomit very severe.

6th.—Projectile vomiting; complete anuria; uræmic coma; convulsions; death 11 A. M.

Autopsy six hours after death; body well nourished; rigor mortis well marked; hypostatic congestion marked in dependent portion of body; integument saffron hue; conjunctivæ yellow; dry omentum; liver pale, boxwood color, very friable; gall bladder filled with bile; left kidney slightly congested with fatty infiltration; right kidney enlarged, much more congested, and less fatty; stomach, numerous extravasations upon mucosa, especially pyloric end, much congestion, serous coat of yellow color; heart and lungs showing steatosis; pericardial sac filled with fluid; spleen normal, bladder empty; cultures from all organs and our bacillus isolated therefrom.

Our studies comprise the cultures obtained from sixty autopsies; from the life blood in five cases; from the exhaled breath in twelve cases; from the sweat or scraping of the surface of the body in twelve cases; from the black vomit in four cases.

Of the sixty autopsies, eight were made and cultures obtained by Dr. John J. Archinard; one was made by Dr. Woodson and Dr. John Archinard at the Jackson Barracks. The other fifty-one were made at the Isolation Hospital. At nearly all of the latter we were present ourselves and made our own cultures. In the first cases, besides taking cultures immediately after section of the body, blood from the cadaver was drawn into sterilized pipettes and large pieces of organs, principally liver, spleen, kidneys, were wrapped up in antiseptic dressings, taken along and incubated for twenty-four or forty-eight hours, according to Sternberg's recommendation; after which, from their interior, cultures were made and animals inoculated. As this inoculated blood and organs did not seem to give us better results, we soon abandoned this procedure.

Our mode of taking cultures at autopsies was as follows: Immediately after section the organs were exposed one at a time, surface cauterized with a piece of flat iron heated to whiteness and a stab made with a sterile, lance-shaped platinum needle; the blood, organ juice, and small *débris* of tissue remaining adherent to the needle were planted on the surface of an agar tube or in lactose bouillon. After a short time bouillon inoculation was done away with on account of the difficulty of transporting same. The cultures brought to the laboratory were then incubated for twenty-four, sometimes forty-eight hours, and then allowed to grow outside of incubator; later on they were plated and the various colonies isolated.

Cultures were taken at each autopsy from the following parts: Peritoneal exudate, pericardial fluid; blood from the heart; blood and juice of the lungs, liver, kidneys, spleen; bile from the gall bladder; excretions from the stomach, and occasionally from other parts.

With the exception of two cases, our cultures always gave us a number of bacteria, and often the plating had to be repeated several times before pure colonies could be obtained. The bacteria found consisted chiefly of our bacilli in association with the coli communis, frequently with the *Proteus vulgaris*, sometimes the *Staphylococcus aureus* and *citreus*, occasially, though rarely, the *Streptococcus pyogenes*. Of the sixty autopsics held, six were rejected, as they did not show the anatomical lesions of yellow fever. In a few other cases little or no growth was obtained from the original culture tubes. In several instances some of the tubes in our laboratory got so mixed up that it was impossible for us to positively identify them, and all these were rejected. On account of these discarded ones the number of autopsies from which cultures were available for our purposes was reduced to thirty-nine. In these thirty-nine cases, we were able to obtain our bacilli thirty-two times; in two cases in a pure condition. In the thirty other cases in association, as stated above. In seven cases we were unable to isolate any bacilli resembling ours.

Five specimens of live blood were obtained from veins at the bend of the elbow, with aseptic precaution from typical cases of yellow fever, on the third, fourth, and sixth days of the disease; six cubic centimetres were taken from each case and mixed up in an Erlenmeyer's flask with five cubic centimetres of sterile lactose bouillon, the whole incubated for forty-eight hours and then plates made therefrom, and a portion used for the inoculation of animals. In two of these cases the growth showed pure cultures of our bacilli; in two other cases. we were able to obtain pure cultures after passing same through animals; in one case the result was negative.

What we call the exhaled breath was obtained by fixing some sterilized cotton in an inhaler and binding same over patient's mouth and nose for from fifteen minutes to an hour; the cotton was afterward planted in lactose bouillon, incubated forty-eight hours, and then plated. In all these cases the patients showed typical symptoms of yellow fever from the fourth to the twelfth day of disease. We were able in twelve such cases to obtain our bacilli only twice.

The sweat and scraping from the body of patients were also investigated. For this purpose a piece of sterilized cotton was rubbed over the face, neck, and upper part of thorax; bathed in perspiration of patients in advanced stages of the disease, and planted in bouillon as above. Of the twelve cases so studied, two gave us the bacilli.

We have used the word bacilli always in speaking of the micro-organism we isolated, because for a while we thought they were two distinct micro-organisms, though morphologically they appeared identical; but some slight variation in their culture development made us feel that it was best to keep them separate. Later experimentations and the passage through animals showed

that we were dealing with one and the same bacillus, for both answered identically to all the culture tests known. Our two organisms we called for convenience' sake bacillus A and bacillus B. Bacillus A was obtained from colonies which appeared nucleated on gelatin plates, whereas bacillus B was obtained from colonies almost identical but not nucleated. Later on we observed, however, that bacillus A, when in the pure state, would as often give rise to non-nucleated colonies as to nucleated ones, and the reverse appeared also for bacillus B. The fact of the matter is, we believe, that at first our cultures of bacillus B were somewhat contaminated with some saprophytic bacteria, and that accounted for the cultural variation which it showed when compared with bacillus A.

In our experiments we obtained bacillus A twenty-nine times; and in four cases we obtained from the same plates both bacilli A and B. The fact noted above, of nucleated and non-nucleated colonies, we have noticed frequently since, when cultivating the *Bacillus icteroides* of Sanarelli on gelatin plates.

The character of our bacillus may be described as follows: A short thick rod measuring from two to four micromillimetres in length by about half the breadth, showing in different media marked pleomorphism; it is very motile, as much so as the *Bacillus typhosus*. It has end flagella very much like this bacillus, stains well in all watery solutions of basic aniline dyes, unstains by Gram's method. It is aerobic—*i. e.*, it grows best in ordinary atmosphere—but is facultative anaerobic; *i. e.*, grows when deprived of oxygen and in the presence of hydrogen. It grows readily in all known neutral or weakly alkaline media, both solid and fluid; it produces very slowly, or not at all, acidity in these media; it grows best at 37° C., but grows also, though more slowly, at ordinary laboratory temperature from 18° to 22° C. In peptone bouillon it grows fairly well, but best in lactose bouillon, causing a general turpidity of the fluid, but no scum and no deposit at the bottom. It causes a slight fermentation in lactose bouillon generally, but occasionally produces no fermentation in this media; in glucose bouillon it produces marked fermentation; in milk it grows readily without producing coagulation even after weeks; in litmus milk it causes a slight acidity, but only very slowly; in Dunham's solution it produces no indol, even after the addition of nitrites, but does so after the addition of an acid; on potatoes it produces a whitish-yellow transparent growth; on blood serum it grows readily, but shows nothing characteristic; on the surface of gelatin it grows readily, showing a whitish transparent growth; on gelatin plates the individual colonies show under the low power of microscope as a gray or yellowish-gray rounded form, iridescent and often containing a nucleus which may be central, but is at times peripheral; this nucleus is generally darker than the rest of the colony and is surrounded by a light halo; in stick culture it grows all along the stab, but more luxuriantly at the surface. It does not liquefy gelatin. On the surface of agar its growth is almost transparent when young. As it grows older it gets whiter. Older colonies at the bottom of the tubes, near the water of condensation, show marked raised points, darker than the rest of the growth, which resemble nuclei. The characteristic growth described by Sanarelli in old cultures of his *Bacillus icteroides* in agar, when growing partly in and partly out of the incubator, has been observed by us, but we have been able to obtain this characteristic growth only when these cultures were quite old. It is typically agglutinated by the blood of yellow-fever patients or of persons who have recently had yellow fever, as will be mentioned later. Inoculated in animals, guinea-pigs and rabbits, it gives rise to typical symptoms, and when inoculated in sufficient quantity causes death, and sometimes very quickly. The pathological lesions found are characteristic, and it is found in the blood and organs of animals dying from its effect, and can be recovered from these in pure cultures.

II. Results of Animal Inoculations.—We give results of injections in guinea-pigs and rabbits, together with post-mortems of these animals. The rabbits were inoculated in the veins of the ears and the guinea-pigs subcutaneously. The material used for inoculation was always a bouillon culture of the bacilli from eighteen to twenty-four hours old, and kept at 37° C.

Rabbit No. 18, weight 1,529 grammes, inoculated with six cubic centimetres bouillon culture of our bacillus (A), died during night of same day; weight after death, 1,470 grammes. Autopsy showed blood-vessels of heart and diaphragm congested; heart full of blood; lungs pale; intestines very much congested; stomach full of food; mucous membrane congested and showing ecchymotic spots; liver fatty and friable; spleen enlarged; gall bladder containing a large quantity of dark bile; kidneys pale and fatty; bladder filled with non-albuminous urine. Cultures made from blood, heart, liver, spleen, showed typical growth of bacillus A.

Pig No. 18, weight 475 grammes, inoculated subcutaneously with twelve cubic centimetres of a lactose bouillon culture of our bacillus A, eighteen hours old, at 37° C., showed shortly afterward signs of depression, temperature rose the next day to 41° C., animal remained quiet and was unwilling to move or eat; he died three days after. Autopsy, immediately after death, showed the blood-vessels of pleura, peritonæum, diaphragm and heart surface very much congested; heart full of dark blood; lungs pale, otherwise normal; liver congested, fatty and friable; kidneys congested and fatty; bladder contained some albuminous urine; stomach and small intestines very much congested with ecchymotic spots in mucous membrane; viscera full of black fluid resembling black vomit. Bacillus A obtained from cultures made of each organ.

Rabbit No. 19, weight 1,990 grammes, injected intravenously with six cubic centimetres of an eighteen-hour-old bouillon culture, our bacillus B; died during the night; post-mortem lesions same as those of rabbit No. 18. Animal was gravid. Cultures as made from all organs and also from fœtus gave us pure cultures of bacillus A.

Pig No. 19, weight 390 grammes, inoculated the same day as pig No. 18, but with bacillus B instead of A; died on the same day as pig No. 18 and showed identical post-mortem lesions, and from all its organs we were able to obtain pure cultures of bacillus B.

Rabbit No. 17, weight 1,950 grammes, inoculated at same time as rabbits Nos. 18 and 19, but with *Bacillus icteroides* of Sanarelli; died the same day as rabbits Nos. 18 and 19 and showed the same lesions; its organs gave pure cultures of the bacillus Sanarelli.

Pig No 17, weight 450 grammes, inoculated with twelve cubic centimetres *Bacillus icteroides* (Sanarelli) on same day as pigs Nos. 18 and 19; died within a few minutes of the latter animals on the fourth day after inoculation, presenting identical post-mortem changes and giving pure cultures from its organs of *Bacillus icteroides.*

Pig No. 20 inoculated with five cubic centimetres of a bouillon culture of bacillus A; became sick, lost appetite, had fever up to 42° C., lost weight, but after three or four days became more lively and began eating again.

Rabbit No. 20 inoculated intravenously with two cubic centimetres bacillus A; lost weight and appetite, was feverish for a few days, afterward recovered appetite and became lively again, and only died thirty-one days after inoculation, showing tuberculosis of lungs and liver. Cultures made from organs and blood remained sterile.

Rabbit No. 21 inoculated intravenously with two cubic centimetres of a bouillon culture of Sanarelli's bacillus; became sick like above, but after a few days recovered and kept alive.

Pig No. 21, inoculated with five cubic centimetres of a bouillon culture of *Bacillus icteroides* (Sanarelli); remained alive after showing for a few days indications of weakness.

III. Comparative Tests between Bacillus Sanarelli and our Bacilli A and B.—The following is a comparative table of our bacilli A, B, and *Bacillus icteroides* of Sanarelli from tests made by us:

Bacillus icteroides (Sanarelli).	Bacillus A.	Bacillus B.
1. Aerobic and facultative anaerobic.	Aerobic and facultative anaerobic.	Aerobic and facultative anaerobic.
2. Short rods 2 to 4.	Short rods 3 to 4.	Short rods 2 to 4.
3. Actively motile.	Actively motile.	Actively motile.
4. Stains readily with aniline dyes; does not resist Gram's method.	Stains readily with aniline dyes; does not resist Gram's method.	Stains readily with aniline dyes; does not resist Gram's method.
5. Does not produce acid in Dunham's solution, even after addition of nitrite; slowly sometimes after addition of acids.	Does not produce acid in Dunham's solution even after addition of nitrite; slowly sometimes after addition of acids.	Does not produce acid in Dunham's solution even after addition of nitrite; slowly sometimes after addition of acids.
6. Slight odor of indol.	Slight odor of indol.	Slight odor of indol.
7 Does not coagulate milk.	Does not coagulate milk.	Does not coagulate milk.
8. Produces acid, but very slowly.	Produces acid, but very slowly.	Produces acid, but very slowly.
9. Ferments glucose rapidly.	Ferments glucose rapidly.	Ferments glucose rapidly.
10. Ferments lactose slightly, sometimes not at all.	Ferments lactose slightly, sometimes not at all.	Ferments lactose more readily.
11. Grows in gelatin, does not liquefy this medium.	Grows in gelatin, does not liquefy this medium.	Grows in gelatin, does not liquefy this medium.
12. Growth on potatoes almost transparent.	Growth on potatoes almost transparent.	Growth on potatoes almost transparent.
13. Produces cloudiness without deposit or scum in bouillon.	Produces cloudiness without deposit or scum in bouillon.	Produces cloudiness without deposit or scum in bouillon.
14. Growth on gelatin whitish transparent; under the microscope (low power) grayish or yellowish-gray, iridescent, and often containing nucleus.	Growth on gelatin whitish transparent; under low power grayish or yellowish-gray, and often contains nucleus.	Growth on gelatin whitish transparent; under low power grayish and sometimes nucleated, at others not.
15. Growth in agar almost transparent when young, porcelain-like when older; at bottom of tubes especially, colonies show marked nucleation when older.	Growth in agar almost transparent when young, porcelain-like when older; at bottom of tubes especially, colonies show marked nucleation when older.	Growth in agar almost transparent when young, porcelain-like when older; at bottom of tubes especially, colonies show marked nucleation when older.
16. Agglutinated by yellow-fever blood.	Agglutinated by yellow-fever blood.	Agglutinated by yellow-fever blood.

Characteristic growth in and out of incubator, as described by Sanarelli, has never shown, except in very old cultures of our bacilli A and B, and the same may be said of the *Bacillus icteroides* grown by us.

Comparative tests to show growth in agar cultures of other bacilli afterward made sterile and culture medium allowed to cool, and then inoculated with our bacilli, and *Bacilli icteroides* of Sanarelli gave us negative results with all. In one or two instances we have noticed that some form of laboratory mold favor the growth of bacillus A, bacillus B, and bacillus Sanarelli.

IV. Serum Reaction with Bacillus Icteroides and our Bacilli A and B.—Pfeiffer's demonstration of the phenomena of agglutination of microbes which consist in the peculiar clumping and stoppage of motion in cultures of before motile bacilli by the addition to those cultures of a small amount of the blood serum of animals previously inoculated with these bacilli, served as a new departure in bacteriology, and enabled us to apply this method for the purpose of aiding in the diagnosis of previously obscure cases.

Widal discovered that the serum of patients afflicted with typhoid fever could agglutinate the *Bacillus ty-*

phosus; he laid down definite and reliable rules for the application of this method to the diagnosis of the disease. Later Wyatt Johnston, of Montreal, showed that a drop of dry blood, afterward properly dissolved and diluted, could be used as efficiently as fresh serum, and we are thus enabled to apply the rules of serum diagnosis to typhoid fever in municipal and board of health laboratories.

Following in those fotsteps we analyzed blood from yellow-fever patients, from healthy individuals, and from cases in various other diseases, for the purpose of applying the Widal test to the *Bacillus icteroides* recently isolated by Sanarelli in yellow-fever cases and to an identical bacillus found by us in a number of 1897 fever cases. The work done in a hundred cases on this subject, in collaboration with Captain R. S. Woodson, M. D., United States army, then stationed in New Orleans, and with our assistant, Dr. John J. Archinard, we submitted to the Orleans Parish Medical Society at its meeting, January 22d, and our paper read then has been published in the February number of the *New Orleans Medical and Surgical Journal*. Since then we have continued and extended our labor, in conjunction with Dr. John J. Archinard, Dr. Woodson having removed to Johns Hopkins Laboratory, and we have added a number of cases to those already observed, which make the results obtained still more interesting and important. A synopsis of all this is here submitted and conclusions drawn therefrom.

We used the blood of fifty cases of yellow fever in the acute stages and during recent convalescence. Twenty of these are of little value to us and should be discarded, as both the bacillus of yellow fever and that of typhoid fever were agglutinated, owing to the fact that the blood was used in too concentrated a form in making the test (one in five, when it should have been one in twenty, or even one in forty). In the remaining thirty cases, when the proper dilution of blood was made (one in forty), we obtained the characteristic clumping of the yellow-fever bacillus in twenty-four cases, a proportion of eighty per cent. If now from these thirty cases we omit three cases where no clumping with the bacillus of the yellow fever was obtained, and a characteristic clumping with the bacillus of the typhoid fever, cases which were thought to be cases of secondary fever following yellow fever, but which were probably simple cases of typhoid fever, our proportion of positive cases would be 88 + per cent. In the yellow-fever cases, omitting the three just referred to, the clumping both of typhoid and of yellow-fever bacilli occurred five times, but four of those cases were cases of yellow fever with a previous history of typhoid, or typhoid cases with a recent history of yellow fever. In only one case did we get such a double reaction without the previous history of typhoid, and in that case it was not possible to ascertain the previous history. In five or six of those cases, where the blood was taken early in the disease (second day), the agglutination was well marked. This seems to point to the fact that the test would be more useful in the early stages of the disease, indeed where it is most needed. Our number of cases has been too few to accept this question as settled. Experiments on a large scale are necessary before concluding finally.

Twenty cases of malarial fever were examined. Plasmodium was found in thirteen cases and not sought for in one case with a distinctive history of malarial fever. In two other cases plasmodium could not be found, though the clinical history was undoubtedly malarial intermittent. Seventeen cases in all, which gave negative results with the *Bacillus icteroides*. In three cases agglutination was obtained; these cases showed no plasmodium, and were all cases of continued malarial fever of five, six, and ten days' duration, occurring in the midst of the epidemic, one of them being an Italian.

Thirty-three cases of typhoid fever were examined; twenty-five cases gave negative results with the *Bacillus icteroides*, and positive results with the *Bacillus typhosus*. In six cases the reaction with both bacilli were obtained; but four of these cases gave histories of recent yellow fever or of yellow fever in 1878. In the other two cases no history of yellow fever could be obtained. In only one case was it positive with the yellow-fever bacillus and negative with the typhoid.

The blood of thirty cases of various diseases were submitted to the test—such as scarlet fever, tuberculosis, acute diarrhœa, alcoholism, chronic dysentery, measles, tertiary syphilis, diphtheria, cancer of the stomach, leprosy, acute jaundice by obstruction, chronic jaundice, nephritis, pneumonia convalescent, cirrhosis of the liver. All gave negative results.

Twenty cases of normal blood coming from individuals who had never had yellow fever, some of them foreigners, gave negative results with the test. This rather limited number, however, was deemed sufficient on account of the uniformity of the results.

To test how long the blood in yellow-fever cases retained its agglutinative power, we tried the blood in ten cases of fever of 1897 who had been well for several weeks, and obtained agglutination in all but one. Also the blood of ten cases of persons who had had well-marked attacks of yellow fever in 1878, and we obtained agglutination with the yellow-fever bacillus in all but one. A number of cases who had had yellow fever in epidemics previous to 1878 (1853, 1867, and 1869) gave us negative results.

Two cases who during the summer had had dengue in San Antonio kindly consented to let us use their blood for our test. In both of them the tests were negative. Ten cases of dengue with eruption, from Galveston, gave us negative results, and will later on apply the test to these.

We can summarize our results as follows:

By the dried-blood method eighty per cent. of the

cases of yellow fever of 1897 agglutinated the *Bacillus icteroides*; nearly twenty per cent. of the cases showed negative. In our typhoid cases with no history of yellow fever the test is almost uniformly negative. In malarial fever whenever plasmodium could be obtained from the blood proving the positive diagnosis of malaria the results of the test were negative. In a number of cases of other diseases the tests were almost always negative. Over ninety cases of normal blood, or blood taken from diseases other than yellow fever, in persons who had never suffered with yellow fever, gave us only four positive results, and indeed in these four cases there is a remote possibility of previous yellow fever.

We would like to emphasize again the fact that our experiments, though pointing to a safe and reliable method of making a diagnosis of yellow fever, do not absolutely settle the question. Experiments should be carried on in a much larger number of cases and by different observers before accepting as final the serum diagnosis of yellow fever.

Conclusions.—1. In a large proportion of autopsies (thirty-two times in thirty-nine) of yellow-fever cases of 1897, in New Orleans, a bacillus was found either in a pure state (two times) or in association (thirty times), similar to the Sanarelli *Bacillus icteroides*. This bacillus has some points in common with the coli communis, but differs from it in some of its essential characteristics.

2. In live blood taken from the veins of the elbow, in well-marked cases of yellow fever, we were able to isolate our bacillus four times in five cases.

3. In the exhaled breath mixed up with secretions from the mouth and nose (sometimes bloody) we isolated our bacillus twice in twelve cases.

4. In the scrapings of the surface of the body of the sick, principally face, neck, in upper part of thorax, we isolated our bacillus two times in every twelve cases.

5. Our bacillus injected intravenously to the rabbit, and subcutaneously to the guinea-pig, in large doses, from five to ten cubic centimetres of a bacillus culture, is always fatal, and sometimes very quickly. In smaller doses (one to two cubic centimetres) the animals are made sick, but generally recover. The animals that die show characteristic lesions of the liver, kidney, and stomach. Cultures from these organs give pure growths of the inoculated bacillus.

6. Our bacillus is identical in almost every respect with Sanarelli's *Bacillus icteroides* obtained from himself, and from Dr. Sternberg, but differs somewhat in its cultural aspects from Sanarelli's description of his bacillus.

7. The blood of yellow-fever cases or of recent convalescents from this disease agglutinates the *Bacillus icteroides* of Sanarelli, and also our bacillus in over eighty per cent. of the cases in the proportion of one part of serum for forty of culture within one hour. In less than twenty per cent. the reaction does not take place.

8. The blood of typhoid and dengue with eruption and malaria fever when properly diluted, 1 in 40, does not agglutinate the *Bacillus icteroides* or our bacillus except in exceptional instances.

9. The blood from a number of diseases other than yellow fever when properly diluted, 1 in 40, does not react on the *Bacillus icteroides* or our bacillus.

10. Normal blood properly diluted, 1 in 40, does not agglutinate the *Bacillus icteroides* or our bacillus.

11. The blood of persons who have had yellow fever seems to retain its agglutinative power for a number of years. The great majority of the cases tested by us who had had yellow fever in 1878 gave the reaction. Those who had had yellow fever previous to 1878 gave us a blood which possessed no agglutinative power with *Bacillus icteroides* or with our bacillus.

PRELIMINARY REPORT ON THE RESULTS OF BLOOD EXAMINATIONS AT CAMP WIKOFF,

AUGUST AND SEPTEMBER, 1898.

By JAMES EWING, M. D.

During the five weeks, August 21 to September 24, 1898, the writer was detailed by Surgeon-General Sternberg to render what assistance blood examinations might give in the diagnosis of tropical and other fevers among the troops from Santiago arriving at Camp Wikoff. A preliminary report of the results of this work has been prepared as follows:

I. Statistical.—The report covers 782 examinations made at Camp Wikoff, seven cases kindly furnished by Dr. Charles Norris, from Swinburne Island, New York city, and eleven cases from miscellaneous sources in New York city. These 800 examinations are all, for the writer's convenience in the subsequent treatment of the material, here considered together.

Of these cases, 605 proved to be of malarial nature. To these may be added 40 cases of typhoid fever developing in malarious subjects, and in which the presence in the blood of pigmented leucocytes and severe anæmia were evidences of recent malarial infection, which was also distinctly indicated by the clinical history.

In the 605 cases of malaria, the plasmodia were found in the blood in 335 cases, while in 270 cases the diagnosis was based upon the clinical history and the discovery in the blood of evidences of malarial infection. These evidences of malarial infection in the blood consisted (1) usually in the presence of intracellular bodies so much affected by quinine that their exact type could not be positively determined; or (2) in the presence of typical pigmented leucocytes; or (3), in chronic cases of distinct clinical character, in the presence of marked anæmia.